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# Prenylated Proteins: Structural Diversity and Functions

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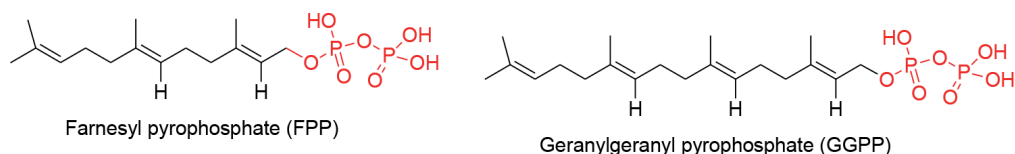
## Abstract

The cell membranes consist of lipid bilayers that are semipermeable. The semipermeable nature enables the cell membranes to regulate the transport of materials entering and exiting the cell. Apart from providing protection and a fixed environment to the cell, the cell membrane has several functions. The covalently linked proteins to lipids on the surface of the cell membranes are the Lipid-anchored proteins. The function of the protein to which the lipid is attached depends on the type of the lipid. Prenylated proteins, fatty acylated proteins, and glycosylphosphatidylinositol-linked proteins (GPI) are the three main types of lipid-anchored proteins on the cell membrane. In particular, the prenylated proteins are very important for cell growth, differentiation, and morphology. The dynamic interaction of prenylated proteins with the cell membrane is important for their signaling functions and is often deregulated in disease processes, such as cancer. An understanding of the prenylated proteins and their mechanisms is important for drug development efforts to combat cancer.

**Keywords:** Prenylation, lipid-anchored proteins, cell signaling, cancer, drug development

## 1. Introduction

The diversity in living organisms is primarily characterized by the proteins found in them. Proteins are large, complex, and diverse in their nature. Their abundance is approximately 50% of the dry tissue weight of the organism. Proteins play several critical functional roles in the body, like providing the structural basis, functional, and regulatory activities in the body's cells, tissues, and organs. Proteins form a very important part of the cell membranes, acting as receptors that trigger specific responses in cells. The semipermeable lipid bilayer of the cell membranes enables the regulation of the materials entering and exiting the cell. On either side of the cell membranes, we can find the lipid-anchored proteins. The lipid-anchored proteins on the surface of the cell membranes have diversity in their function depending not only on the type of the lipid covalently attached to the protein but also on the site where the lipid binds to the protein. The three main types of lipid anchored proteins on the cell membrane are prenylated proteins, fatty acylated proteins, and glycosylphosphatidylinositol-linked proteins (GPI). These proteins play a very important role in cell interactions due to the diversity of the lipid groups attached to them. The lipid groups



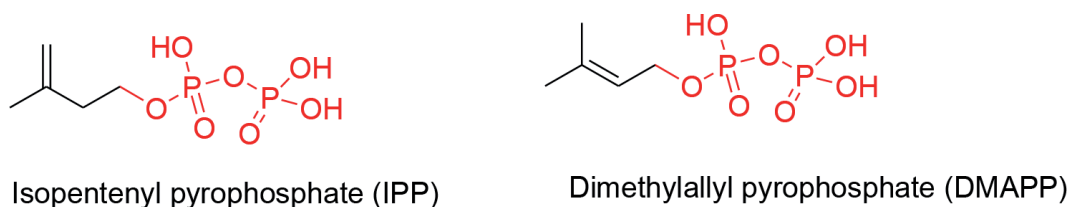
**Figure 1.**  
*Prenylation substrates.*

alter the molecular hydrophobicity and allow the interaction of cell membranes and protein domains with the lipid-anchored proteins [1].

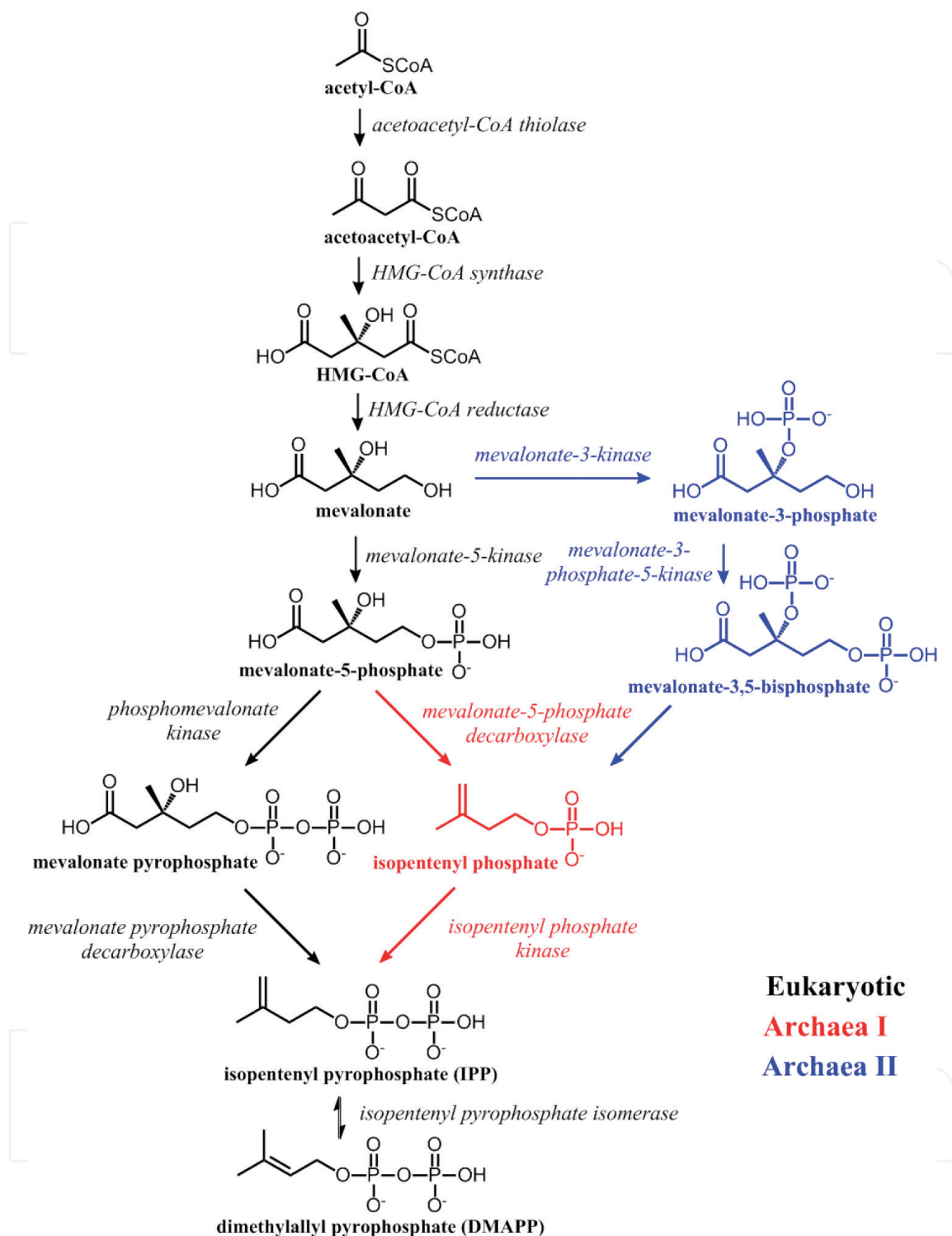
Proteins are formed as a result of the translation of the nucleic acid sequence to polypeptide sequence. But this simple translation is insufficient to cater to the ever-expanding need for the protein functions necessitated by the complex environmental stimuli [2]. The organisms have thus evolved a vast repertoire of co-translational and post-translational modifications to the protein architecture. Prenylation and/or lipidation are the major post-translational modifications that control the localization and activity of several proteins having crucial regulatory functions. Critical changes in the structure and physicochemical properties of proteins are brought about by the attachment of the lipid groups to the protein. This modification affects the cellular localization, stability, and biological activity of the modified proteins. The lipid modifications in proteins were first discovered by Folch in 1951 [3]. A mating factor in fungus *Rhodospiridium toruloides* was found to be S-farnesylated peptide, a class of prenylated protein in 1978 [4]. Nuclear lamin proteins namely, B lamins and prelamin A were the first prenylated proteins that were identified in mammalian systems [5, 6]. Since then several proteins and peptides known to be modified with prenylation are reported and their number is increasing. Prenylation has been studied extensively owing to its role in the function of the proteins in several cellular activities. Prenylation of proteins include the farnesylation and geranylgeranylation, with the farnesyl pyrophosphate (FPP) or geranylgeranyl pyrophosphate (GGPP) as substrates respectively (**Figure 1**). These hydrophobic prenyl groups anchor the proteins to the cellular membranes and can trigger several cells signaling pathways.

## 2. Prenylation: its mechanism

The five-carbon (C5) isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP) (**Figure 2**) are termed as isoprene units are the building blocks of isoprenoids also called as terpenoids. These lipophilic molecules are ubiquitous and exist in all living organisms. They represent the largest and most diverse group of natural compounds [7]. Thousands of isoprenoids are synthesized by



**Figure 2.**  
*Building blocks of isoprenoids.*



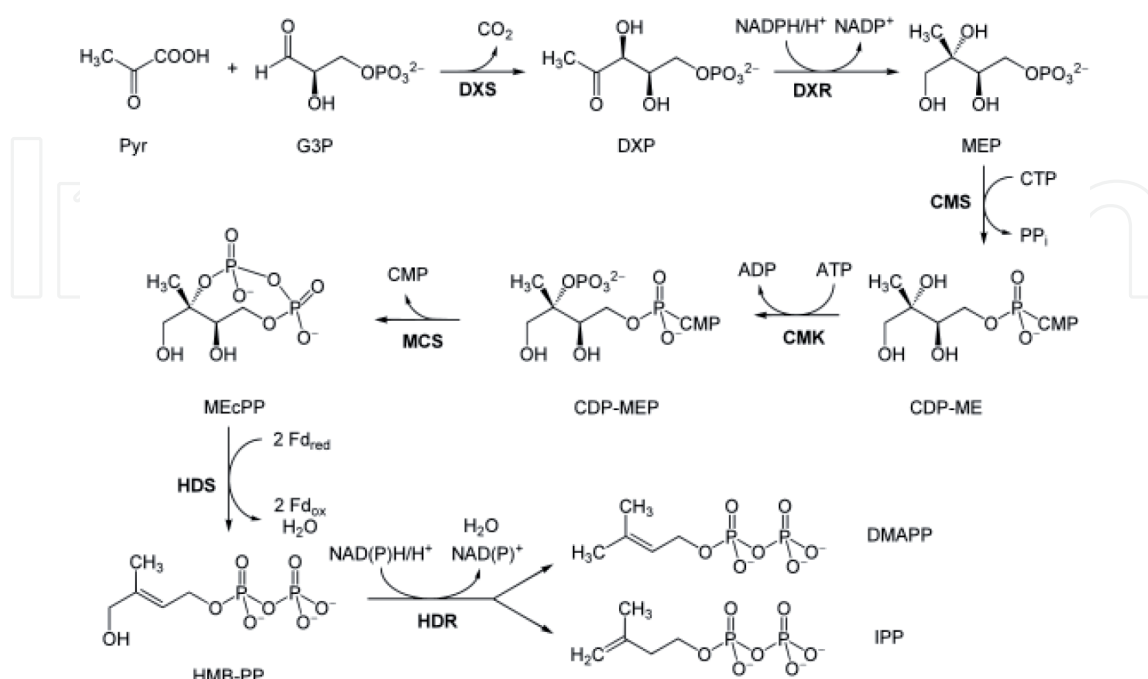
**Figure 3.** Mevalonate pathway. This file is licensed under the Creative Commons Attribution-Share Alike 4.0 International.

“head to tail” or “head to head” addition of either IPP or DMAPP. All living organisms can synthesize isoprenoids. But the mevalonate (MVA) pathway (**Figure 3**) is exclusively used by animals, fungi, and archaebacteria for the synthesis of isoprenoids [8]. A series of reactions beginning with the conversion of acetyl-CoA to MVA and further to IPP and DMAPP constitutes the MVA pathway. With the synthesis of IPP and DMAPP, further head to tail condensation of two IPP molecules with DMAPP gives FPP (C15) catalyzed by Farnesyl pyrophosphate synthase (FPS; EC 2.5.1.10).

Subsequent addition of another molecule of IPP to FPP catalyzed by GeranylGeranyl diphosphate synthase (GGPPS; EC 2.5.1.29) gives GGPP(C<sub>20</sub>). The enzymes involved in the conversion of acetyl-CoA to IPP and DMAPP have been identified in peroxisomes [9] as well as in the cytosol [10].

The biosynthesis of the isoprenoids was once thought to occur exclusively by the MVA pathway. But the identification of a non-MVA pathway in plants and micro-organisms was suggested by the experimental data and this pathway was named as methylerythritol 4-phosphate pathway (MEP) after the precursor (**Figure 4**) [11, 12]. While most eubacteria and *Plasmodium falciparum*, the malarial parasite uses the MEP pathway, archaeobacteria, fungi, and animals use the MVA pathway to synthesize the isoprenoids. It is the plants that use both the pathways albeit in different compartments [13, 14]. Since the majority of the pathogenic bacteria employ the MEP pathway and the same is missing in the mammalian system, the enzymes of the MEP pathway are potent targets for the new anti-infective drugs [15]. In recent years following the discovery that FPP and GGPP could modify the structure and function of unique groups of proteins, which are implicated in cancer and other disorders, through the process of protein prenylation, the interest in isoprenoids and related pathways has increased substantially [16].

The attachment of a lipophilic isoprenoid group covalently to a molecule is referred to as prenylation. FPP and GGPP, generally referred to as prenyl groups are the most relevant isoprenoids relevant to protein prenylation. Protein prenylation is an irreversible covalent post-translational modification comprising of the addition of FPP or GGPP covalently to the cysteine residue at the C-terminus of the protein. Protein prenylation through a thioether linkage (C-S-C) is a relatively stable bond. The cysteine residue located at the C-terminus consists of “CAAX” consensus sequence, with C being cysteine, A being any aliphatic amino acid except alanine, and X being serine, alanine, methionine, or glutamine in case of farnesylation and X being leucine or isoleucine, in case of geranylgeranylation. Either farnesylation or geranylgeranylation



**Figure 4.** MEP pathway reactions in the biosynthesis of isoprenoids. Redrawn verbatim from the scheme of Qidwai and coworkers “Exploring Drug Targets in Isoprenoid Biosynthetic Pathway for *Plasmodium falciparum*.” *Biochemistry Research International*. 2014: 657189.

C terminal consensus sequence of amino acids	C	A	X	Prenylation
CAAX	cysteine	any amino acid except alanine	serine, alanine, methionine, or glutamine	farneysylation
			leucine or isoleucine	geranylgeranylation
			phenylalanine	Either farnesylation or geranylgeranylation

**Table 1.**  
*Consensus sequences and prenylation.*

is possible if X is phenylalanine. Thus “X” residue is largely responsible for determining which isoprenoid is attached to the protein target **Table 1** [17].

Farnesyltransferase (FTase), geranylgeranyltransferase type 1 (GGTase-I) Geranylgeranyltransferase type 2 (GGTase-II), and Geranylgeranyltransferase type 3 (GGTase-III) are the enzymes collectively referred to as the prenyltransferases. These prenyltransferases catalyze the addition of the prenyl groups to the proteins which is crucial for their activities **Table 2**.

FTase and GGTase-I are  $\alpha\beta$  heterodimeric proteins and share a common  $\alpha$ -subunit (FNTA) and different  $\beta$ -subunits (FNTB and PGGT1B, respectively) [18, 19]. GGTase-II is also a heterodimer, having distinct  $\alpha$  and  $\beta$  subunits (RABGGTA and RABGGTB, respectively). A third subunit denoted as Rab escort protein (REP) forms a very important functional part of GGTase-II [20]. The cytosolic fraction of all tissues including the brain contains all these three enzymes. The active sites of FTase and GGTase-I completely cover the isoprenoid and major parts of the C-terminus of the substrate protein and their three-dimensional structures exhibit high similarities. Recent reports point out that the FTase can prenylate cysteine residues efficiently at C-terminus position number five instead of the usual four [21]. This raises the possibility of the number of prenylated proteins to be larger than the one already envisaged. FTase is more selective compared to GGTase-I. While GGTase-I accepts many of the FTase substrates, the converse is generally not true with very few exceptions [22].

In addition to the CAAX, GGTase-II can also recognize CC, CXC, CCX, CCXX, and CCXXX at the C-terminus [23, 24]. Both cysteine residues within the sequences noted above are geranylgeranylated by GGTase-II.

The substrate specificities determined for GGTase-I and GGTase-II suggested that a third type of enzyme GGTase III may be found to catalyze the geranylgeranylation of the CXC terminating proteins [25, 26]. GGTase-III consists of prenyltransferase  $\alpha$

Prenylation reaction	Enzyme	Examples of prenylated substrates
Farnesylation	FTase	Ras proteins, lamin A, lamin B, CENP-E, CENP-F, transducin $\gamma$ subunit
Geranylgeranylation	GGTase I	Rho proteins, Rac proteins, Ral proteins, Rap proteins, $G\gamma$ subunit
	GGTase II	Rab proteins
	GGTase III	Ykt6, FBXL2

**Table 2.**  
*Prenyl-modifying enzymes and their protein substrates.*

subunit repeat-containing 1 (PTAR1) and the  $\beta$  subunit of RabGGTase. GGTase-III has been identified using a biotinylated geranylgeranyl analogue, the Golgi SNARE protein Ykt6, and ubiquitin ligase FBXL2 as a substrate. GGTase-III transfers a geranylgeranyl group to mono-farnesylated Ykt6, generating doubly prenylated Ykt6 [27].

Generally, the proteins after prenylation with few exceptions, get localized in the endoplasmic reticulum for further processing [28]. The two proteases namely Ras converting enzyme (Rce1) or Zinc metalloproteinase Ste24 (ZMPSte24) cleave the “AAX” residues from the CAAX-box on farnesylated proteins [29, 30].

## **2.1 Farnesylation**

Zinc is used to coordinate the binding of farnesyl moiety to the thiol or sulfhydryl group of the cysteine in the CAAX box, thus pointing to the fact that FTase is a metalloenzyme. The FPP is bound by the FTase first, which is then followed by the binding of the protein. The release of the farnesylated protein occurs only after the farnesyl moiety is transferred to the protein. Farnesylation is a very essential modification in proteins to enable them to bind to membranes and thereby mediate signal transduction, both in normal as well as malignant cells [31].

Farnesylated proteins need a second signal for binding to the membrane since the farnesyl moiety is not sufficiently hydrophobic to stably anchor a protein to the membrane. The essential second signal is either in the form of one or two palmitate moieties attached to the cysteine upstream to the farnesylated cysteine residue or a polybasic K-Ras4B motif [32]. The movement of farnesylated K-Ras4B in the cytosol of the cell is regulated by the farnesyl electrostatic switches [33].

## **2.2 Geranylgeranylation**

Unlike the farnesyl moiety the geranylgeranyl moiety is sufficiently hydrophobic. This makes the membrane binding of the modified protein more stable. A mechanism to extract the geranylgeranylated Rab and Rho proteins from the membrane using protein-protein interactions is observed, though the geranylgeranyl moiety is not removed from the modified protein all through its lifetime. The GTP-bound forms of Rab and Rho proteins are bounded to the soluble Guanine Dissociation inhibitor (GDI) proteins. The hydrophobic cavity of the GDI binds to at least one isoprenoid moiety. The inactivation of the activated Rab and Rho in the cytosol is preceded by their removal from the membrane as a result of isoprenoid sequestration [32].

## **3. Diversity of prenylated proteins and their functions**

A large number of proteins have been identified which have undergone prenylation including several important intracellular proteins like heterotrimeric G protein subunits, nuclear lamins, Ras superfamily of small GTPases comprising of more than 150 known members [34]. This superfamily is divided into five subfamilies based on sequence and functional similarities: Ras, Rho, Rab, Ran, and Arf [35, 36]. During signal transduction, these proteins act as molecular switches cycling between “on” and “off” states. They regulate a variety of cellular functions like growth, differentiation and proliferation, vesicular trafficking by interacting with the downstream effectors. Each subfamily interacts with multiple effector proteins downstream. The

activation of the downstream signaling pathway is dependent on its association with the cellular membrane which in turn relies on prenylation [37]. The prenylation of the small GTPases impacts its structure and function and in turn, affects the functions of the effector proteins downstream.

### **3.1 Nuclear lamins**

The major architectural proteins of the animal cell nucleus are constituted by the lamins, which line inside the nuclear membrane and provide a platform for the binding proteins. They belong to the intermediate filament (IF) family of proteins and constitute an important part of the cytoskeleton. Based on the genomic structure and nucleotide sequence the nuclear lamins represent type V of the six types of IF superfamily. Based on the structural and protein features and expression patterns the nuclear lamins are divided into A and B types. Type A lamins are generally expressed in developmentally regulated temporal patterns but the Type B is expressed ubiquitously. Lamins contain a CAAX box at the C-terminus with few exceptions. These CAAX boxes serve as sites of post-translational farnesylation. The isoprenylation of the cysteine is followed by the proteolytic cleavage of the AAX motif. Carboxy methylation of cysteine is the last of the three successive steps in the modification. After the cell division, the nuclear reassembly is mediated by the lamins. Type A lamins are distributed in the cytoplasm as they are easily solubilized whereas Type B lamins are closely associated with the nuclear membrane. The differential farnesylation of the lamin protein leads to their differences in membrane attachment during mitosis. During mitosis, the mature Type B lamins are anchored to the membrane by the farnesyl moiety and the Type A lamins are more soluble in the cytoplasm as the farnesyl moiety is removed [38].

### **3.2 Ras superfamily of small GTPase**

The H-Ras, K-Ras, and N-Ras are the best-studied Ras isoforms owing to their oncogenic roles. They undergo the three-step modification namely prenylation, proteolysis, and carboxymethylation at the membrane of the endoplasmic reticulum. The important biological functions of Ras-like interaction with regulatory proteins, effector protein downstream including the signal transduction occur at the plasma membrane. Regulated by sophisticated mechanisms, the Ras proteins switch between inactive GDP-bound or active GTP-bound forms at the plasma membrane. In response to the stimulation by the extracellular signals through the cell surface receptors, the Ras proteins are switched on by guanine nucleotide exchange factors (GEFs), which catalyze the release of GDP and the binding of GTP. The Ras proteins are switched off by the GTPase-activating proteins (GAPs) which accelerate the intrinsic GTPase activity of Ras for hydrolysis of GTP. This Ras GDP/GTP switch is further regulated by guanine dissociation inhibitors (GDIs) and GDI-like proteins. A conformational change in Ras proteins is induced by the replacement of GDP for GTP which allows them to interact with their downstream effectors and execute their multiple signaling functions [39]. The selective interaction of K-Ras with membrane lipids leads to the formation of “nanoclusters” that determine its signaling output. The lipid specificity and signaling output of the K-Ras can be altered by subtle changes to its membrane anchor sequence or prenylation [39]. The importance of the prenylation in the functional regulation of Ras renders the isoprenoids and prenyltransferases as viable targets for therapeutic purposes.



## **4. Prenylated proteins and their functional regulation**

The intracellular GTP-binding proteins are the largest family of prenylated proteins. They transduce extracellular signals into intracellular changes via downstream effectors. These proteins include the heterotrimeric G protein subunits and the small G protein superfamily. Despite the fact that both heterotrimeric and small G proteins have GDP/GTP-binding and GTPase activity only the small G proteins are commonly referred to as GTPases. The small G proteins are monomeric proteins and they are structurally and functionally distinct from the heterotrimeric G proteins. The heterotrimeric G proteins are activated by agonist-bound G protein-coupled receptors (GPCRs). GTP exchange on small G proteins is controlled by GEFs that catalyze the exchange of GDP for GTP. The functions of many of the small G proteins can be classified broadly as follows: regulation of gene expression by Ras subfamily; regulation of both cytoskeletal reorganization and gene expression by the Rho/Rac/Cdc42 proteins of the Rho family; regulation of intracellular vesicle trafficking the Rab and Sar1/Arf proteins, and regulation of nucleocytoplasmic transport during the G1, S and G2 phases of the cell cycle the Ran family members [40].

### **4.1 Guanine dissociation inhibitors (GDIs)**

The pivotal regulators of small G protein function are the GDIs. The activation of the G proteins is prevented by Rho GDIs via three distinct mechanisms. The isoprenoid group of the GTPase is inserted into the hydrophobic pocket formed by the immunoglobulin-like  $\beta$  sandwich of GDI and thus shielded from the solvent. This enables the GDIs to maintain the Rho GTPases as soluble cytosolic proteins forming high-affinity complexes. The dissociation of GDP from Rho proteins is inhibited by GDIs, preventing GTPase activation by GEFs. Finally, the GDIs are able to interact with the GTP-bound form of the GTPase to prevent interactions with effector targets [41, 42].

### **4.2 Guanine nucleotide exchange factors (GEFs)**

After dissociation from the GDIs, an extremely slow process catalyzed by the GEFs is the association of small G proteins with plasma membranes and exchange of GDP for GTP. The general mechanistic features of GEF-catalyzed GDP dissociation from Rho family proteins appear to be conserved for Ras family proteins and their GEFs. The GDP-GTP exchange reaction is thought to be the rate-limiting step in the GTP-binding/GTP hydrolytic cycle of GTPases and the GEFs have therefore are the key regulators of the GTPases.

### **4.3 GTPase-activating proteins (GAPs)**

The action of the intrinsic GTPases converts GTP-bound forms of the GTPases to the inactive GDP-bound forms. Hydrolysis of GTP by intrinsic GTPases occurs slowly under normal circumstances, but a further group of regulatory proteins known as GAPs can accelerate this process. These proteins have important and widespread roles in the regulation of the small GTPases. GAPs are classified according to their GTPase subfamily (Ras-GAP, Rap-GAP, etc.) with sequence homology within subfamilies but not between families [43].

## 5. Prenylation and cancer

The prenylated proteins are ubiquitous in nature and have diverse roles in cellular biology. Detailed research on the oncogenic potential of prenylated proteins has been stimulated by the fact that mutations within the Ras family could be identified in as many as 10–15% of all human cancers [44, 45]. New insights into the role of farnesylated proteins in neoplasia have been provided by the use of farnesyl transferase knockout mouse by showing that farnesylated proteins apparently are not required for the initial malignant cellular transformation but are critical for tumor progression and maintenance. Of the GTPase regulatory proteins, a number of GEFs have also been associated with human malignancy. A gene rearrangement of GEF gene has also been identified in a patient with acute myelogenous leukemia. The drug discovery endeavors are directed towards the development of farnesyltransferase inhibitors (FTIs) as novel anticancer drugs owing to the central role of prenylated proteins in malignancy [46–48]. Many CAAX peptidomimetics, as well as FTIs and geranylgeranyltransferase inhibitors (GGTIs) have been developed that are highly potent and with low toxicity [49, 50]. The anchorage independent growth of cancer cells is inhibited by FTIs causing changes in cell cycle progression which further induce the apoptosis of cancer cells and inhibition of their attachment to extracellular substratum [51]. It has been shown that FTIs can also inhibit GGTase-II along with FTase, resulting in the blocking of post-translational modification of Rab proteins, leading to cell death [52]. This interference with endosomal trafficking is attributed as a novel action of FTIs. A number of prenylated proteins have been shown to be involved in the initiation, invasion, and progression of cancer in addition to the Ras proteins [53]. A search for important enzymes within the prenylation pathway as potential anticancer therapeutics is underway. Prenylated proteins also appear to play a role in both benign and malignant bone disease. The bisphosphonates are drugs that inhibit farnesyl diphosphate synthase resulting in effects on osteoclasts and tumor cells. Inhibition of protein prenylation and Ras signaling within osteoclasts leads to defects in intracellular vesicle transport that result in the imperfect formation of the tight-sealing zones or ruffled borders required for bone reabsorption [54]. Aside from their antiproliferative effects via inhibition of Ras, bisphosphonates also appear to have important proapoptotic effects that may be mediated by the accumulation of isopentenyl diphosphonate. Isopentenyl diphosphonate can be metabolized to Apppi (triphosphoric acid 1-adenosine-5'-yl ester 3-[3-methylbut-3-enyl] ester), an intracellular ATP analogue that can directly induce apoptosis [55]. To suppress the activity of the oncogenic Ras proteins to achieve the antitumor activity, the inhibition of prenylation and related pathways are being extensively investigated.

## 6. Conclusion

Prenylation is an irreversible post-translational modification during the lifetime of the protein. It is responsible for the correct cellular localization, activity, and protein–protein interactions of a number of signaling proteins. Over the years, the structural and mechanistic features of the prenyltransferase enzymes have been probed using a large number of isoprenoid analogs. The enzymology of prenyltransferases is well understood with extensive studies using these analogs. The malignant activity of oncogenic Ras proteins can be suppressed by using prenyltransferase inhibitors. But much remains to be learned concerning the roles of prenylated proteins in living cells,

and this remains an intense area of current investigation. Site-specific modifications of proteins are a new possibility with prenylation. Overall, the challenges in the investigation of protein prenylation will keep this vibrant field of inquiry an active exciting endeavor in the near future.

### **Conflict of interest**

The authors declare no conflict of interest.

### **List of Abbreviations**

GPI	Glycosylphosphatidylinositol-linked proteins
FPP	Farnesyl pyrophosphate
GGPP	Geranylgeranyl pyrophosphate
FPS	Farnesyl pyrophosphate synthase
GGPPS	Geranylgeranyl diphosphate synthase
IPP	Isopentenyl pyrophosphate
IF	Intermediate Filaments
DMAPP	Dimethylallyl pyrophosphate
MVA	Mevalonate
MEP	Methylerythritol 4-phosphate pathway
FPS	Farnesyl pyrophosphate synthase
GGPPS	Geranylgeranyl diphosphate synthase
FTase	Farnesyltransferase
GGTase-I	Geranylgeranyltransferase type 1
GGTase-II	Geranylgeranyltransferase type 2
GGTase-III	Geranylgeranyltransferase type 3
FNTA	FarNesylTransferase, Alpha subunit
FNTB	FarNesylTransferase, Beta subunit
PGGT1B	Protein GeranylGeranylTransferase type I subunit Beta
RAB	Ras-associated binding
Ras	Rat sarcoma
RABGGTA	Rab Geranylgeranyltransferase Subunit Alpha
RABGGTB	Rab Geranylgeranyltransferase Subunit Beta
REP	Rab escort protein
PTAR1	Prenyltransferase $\alpha$ subunit repeat containing 1
Rce1	Ras converting enzyme
ZMPSte24	Zinc metalloproteinase Ste24
GEFs	Guanine nucleotide exchange factors
GDI	Guanine dissociation inhibitors
GPCRs	G protein coupled receptors
GAPs	GTPase-activating proteins
FTIs	Farnesyltransferase inhibitors
GGTIs	Geranylgeranyltransferase inhibitors

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